DRUG FORMULATIONS AND CLINICAL METHODS

Validation of a Capillary Electrophoresis Method for the Simultaneous Determination of Amlodipine Besylate and Valsartan in Pharmaceuticals and Human Plasma

AHMED O. ALNAJJAR
King Faisal University, College of Science, Department of Chemistry, PO Box 400, Al-Ahssa 31982, Saudi Arabia

A rapid, simple, and sensitive capillary electrophoresis (CE) method was developed and validated for the simultaneous determination of amlodipine (AML) and valsartan (VAL) in pharmaceuticals and human plasma using a UV photodiode array detector. Electrophoretic conditions were optimized to improve separation, sensitivity, and rapidity. The optimal conditions were 25 mM phosphate buffer at pH 8.0, injection time 10.0 s, voltage 25 kV, and column temperature 25°C, with detection at 214 nm. The method was found to be linear in the range of 1.0–35 and 1.0–350 mg/L, with weighted regression 0.9999 and 0.9994, for AML and VAL, respectively. Validation of the method showed acceptable intraday and interday accuracy (85.5–95.3%) and precision (RSD 1.64–4.2%) in pharmaceutical formulation and human plasma analysis. The sensitivity of the method was enhanced by both optimization of the CE procedure and preconcentration performed by liquid–liquid extraction. The LOD for both AML and VAL was 0.03 mg/L, which allows analysis at the level of the drugs possibly found in human plasma. Therefore, the proposed method is suitable for QC in pharmaceutical laboratories and therapeutic drug monitoring in clinical laboratories.

Amlodipine (AML) besylate, 3-ethyl 5-methyl 2-[(2-aminoethoxy)methyl]-4-(2-chlorophenyl)-6-methyl-1,4-dihydropyridine-3,5-dicarboxylate benzenesulfonate (Figure 1), is a long-acting calcium channel blocker. It is frequently used as an antihypertensive agent and in the treatment of angina. Like other calcium channel blockers, AML acts by relaxing the smooth muscle in the arterial wall, decreasing peripheral resistance and, hence, reducing blood pressure; in angina, it increases blood flow to the heart muscle (1).

Valsartan (VAL) is chemically named *N*-(1-oxopentyl)-*N*[2′-(1*H*-tetrazol-5-yl)[1,1-biphenyl]-4-yl]methyl]-L-valine (Figure 1). It is an angiotensin II receptor antagonist, acting selectively on the angiotensin type II receptor, which is responsible for vasoconstriction, and prevents salt and water retention (2).

Previously, AML and VAL were given individually to keep blood vessels from narrowing, which would decrease blood pressure and improve blood flow. Recently, they were prepared in a combination. The new formulation is used to treat hypertension. Therefore, analytical methods for their separation and quantification in pharmaceutical formulations and in human plasma are desirable for QC and therapeutic drug monitoring, respectively. Review of literature revealed that no assay method is available for the simultaneous determination of these two drugs in biological fluids. However, spectrophotometric (3), voltammetric (4), HPLC (5), and TLC (6) methods were recently published for their simultaneous determination in pharmaceutical preparations. Other methods have been published for single-component determination, in combination with other drugs, or of AML and VAL in pharmaceutical preparations and/or biological fluids, including spectrophotometry (7, 8), HPLC (9–15), TLC (16), and capillary electrophoresis (CE; 17, 18).

It is well known that pharmaceutical and clinical analyses depend mainly on LC. However, CE was introduced as an alternative technique for LC. The advantages of CE over LC are greater separation efficiency, shorter analysis time, and lower consumption of reagents and samples (19). Moreover, CE analysis provides reduction in sample preparation, simplicity in instrumental operation, and good ruggedness. These advantages allow CE to be applied with a single set of operating conditions to a wide variety of analytes. Therefore, the benefits of CE empower the technique with great utility to be applied in pharmaceutical and clinical analyses.

The aim of this study was to develop a CE methodology for simultaneous determination of AML and VAL in pharmaceutical formulations and human plasma. Attention was focused on the development of the major CE features, including separation, sensitivity, and rapidity, by optimizing the electrophoretic factors potentially controlling the analysis, including electrolyte concentration, pH, separation voltage, injection time, and column temperature.

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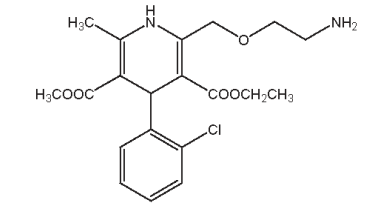
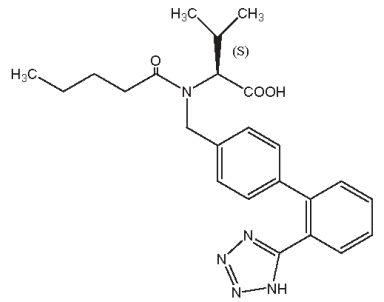
Corresponding author's e-mail: anajjar@kfu.edu.sa



Amlodipine Besylate



Valsartan

Figure 1. Chemical structure of amlodipine besylate and valsartan.

Experimental

Chemicals, Reagents, and Pharmaceuticals

All chemicals and reagents used in this study were of analytical grade. VAL was generously donated by Saudi Pharmaceutical Industries & Medical Appliances Corp. (SPIMACO, Qassim, Saudi Arabia). AML, acetic acid, boric acid, phosphoric acid, sodium acetate, sodium tetraborate decahydrate, and sodium phosphate were supplied by Sigma-Aldrich (St. Louis, MO).

Amlor® (5 and 10 mg AML) and Diovan® tablets (40, 80, 160, and 320 mg VAL) were manufactured by Pfizer (New York, NY) and Novartis (Basel, Switzerland), respectively. Exforge® tablets (5 and 10 mg AML; 80 and 160 mg VAL) were also manufactured by Novartis.

Instrumentation and Software

A P/ACE MDQ CE system coupled with a photodiode array detector (PAD) supplied by Beckman (Fullerton, CA) was used throughout the experiments. Separation was carried out in a 47 cm long (40 cm to the detector) × 50 μm id fused-silica capillary housed in a cartridge with a detector window, 100 × 800 μm (10 cm to the detector, short way).

After each experiment, the capillary was washed with 0.1 M sodium hydroxide for 1.0 min and the running buffer (25 mM sodium phosphate adjusted to pH 8.0 with phosphoric acid). The hydrodynamic injection mode was applied for sample loading.

32 Karat version 7.0 software supplied from Beckman was used to control the CE system as well as for data acquisition and processing.

Preparation of Standard Solutions

Standard stock solutions at concentrations of 1 mg/mL AML and 16 mg/mL VAL were prepared by dissolving appropriate amounts of the standard drugs in a few drops of methanol and then making up to volume with the running buffer. Calibration curves were prepared by taking appropriate aliquots of AML and VAL standard stock solutions in different 10 mL volumetric flasks and diluting up to the mark with the running buffer to obtain final concentrations of 1.0, 5.0, 10, 20, 30, and 35 mg/L and 1.0, 10, 50, 100, 200, and 350 mg/L, respectively. All solutions were stored at 4°C and equilibrated to room temperature before use. Calibration curves were constructed by plotting average peak area against concentration, and regression equations were computed for AML and VAL.

Preparation of Pharmaceutical Samples

Twenty Exforge tablets were powdered. An amount equivalent to 10 mg AML and 160 mg VAL was weighed and dissolved in few drops of methanol and then in the running buffer in a 25 mL volumetric flask. The volume was made up to the mark, and the solution was filtered through a 0.45 μm PTFE membrane filter. The filtered solution was diluted with running buffer to obtain final concentrations of 0.1, 10, and 30 mg/L for AML and 10, 100, and 300 mg/L for VAL and then analyzed under the optimized electrophoretic conditions.

Other pharmaceutical samples were formulated in our laboratories, including AML and VAL in different concentrations with excipients (microcrystalline cellulose, magnesium stearate, starch, and talc) usually found in tablet formulations. In addition, placebo samples were prepared including the same excipients.

Preparation of Human Plasma Samples

The extraction procedure used for the preparation of human plasma samples was a modification of our previous procedure (20). Blood samples were collected from healthy, drug-free volunteers. Samples were spiked with three appropriate volumes of standard solutions of both drugs to give final concentrations of 0.1, 0.15, and 0.2 mg/L for AML and 0.1, 0.2, and 0.3 mg/L for VAL, which were values possibly found in human plasma. After that, samples were centrifuged at 4600 rpm for 5 min. The drugs were extracted using the following liquid–liquid extraction (LLE) technique. To each tube containing 1.0 mL sample, 5 mL acetonitrile was added and vigorously vortex-mixed for 1 min. Samples were centrifuged again, and the organic phase was transferred to another tube and evaporated to dryness under a stream of nitrogen gas. The residue was first dissolved in 1.0 mL methanol; then 99.0 μL running buffer was added using micropipets.

Table 1. Intraday precision and accuracy of the proposed method for analysis of synthesized pharmaceutical formulation and spiked human plasma samples

	Concn added, mg/L	Concn found, mg/L					Mean, mg/L	±SD	Precision, RSD, %	Accuracy, %
					Pharmaceutical samples					
AML	1.00	0.93	0.93	0.89	0.95	0.87	0.91	±0.03	3.60	91.40
	10.0	8.80	8.85	9.21	9.11	9.47	9.09	±0.27	3.02	90.88
	30.0	27.33	27.70	28.52	28.81	29.30	28.33	±0.81	2.85	94.44
VAL	10.0	8.48	9.25	8.87	8.65	9.10	8.87	±0.32	3.56	88.70
	100	90.88	93.86	92.83	93.85	94.93	93.27	±1.53	1.64	93.27
	300	285.88	297.92	280.95	280.89	283.81	285.89	±7.04	2.46	95.30
					Spiked human plasma samples					
AML	0.100	0.087	0.085	0.090	0.089	0.088	0.088	±0.002	2.19	87.80
	0.150	0.128	0.130	0.125	0.138	0.129	0.130	±0.005	3.73	86.67
	0.200	0.173	0.172	0.184	0.174	0.180	0.177	±0.005	2.93	88.30
VAL	0.100	0.082	0.090	0.085	0.089	0.089	0.087	±0.003	3.90	87.00
	0.200	0.173	0.182	0.175	0.173	0.178	0.176	±0.004	2.18	88.10
	0.300	0.265	0.285	0.279	0.269	0.265	0.273	±0.009	3.30	90.87

Results and Discussion

Preliminary Investigation

Spectrum scanning for AML and VAL was performed individually in the range of 190–400 nm using the PAD. The highest absorbance for both drugs was obtained at 214 nm; therefore, this wavelength was used for further measurements.

The stability of AML and VAL in stock solutions was examined. The results showed that no significant degradation occurred when solutions were stored at 4°C for 72 h.

Method Optimization

The aim of the optimization of the proposed CE method was to obtain efficient separation, good detectability, and short analysis time. Electrophoretic factors potentially affecting these features, including electrolyte concentration, pH, injection time, and separation voltage, were optimized.

The most common range of voltage used in CE (5–30 kV) was examined in this study (21). Higher voltage reduced analysis time, but some loss in separation occurred. Shorter analysis time and better separation was obtained at 25 kV, which was adopted for further optimization studies.

A 1.0–20.0 s range of the injection time (21) was examined at pressure 0.5 psi. Longer injection time improves the signal but causes some loss in resolution and peak symmetry. A 10 s injection time gave better results and was used as the optimum value.

The practicable range of column temperature from 5 to 30°C was tested (21). The temperature had no significant effect on the responses; therefore, the normal temperature (25°C) was set for further analyses.

Different concentrations of phosphate buffer in the range of 10–100 mM were examined. For concentrations up to 100 mM, the resolution and analysis time increased with ionic strength. The optimum electrolyte concentration adopted was 25 mM, which gave acceptable resolution and a short analysis time. To optimize the pH of the buffer electrolyte, a range of pH 2.5–9.0 was applied. A pH value of 8.0 proved to be ideal for separation. At lower pH the reduction of the electro-osmotic flow increased the analysis time and resulted in a loss of efficiency. On the other hand, at a pH higher than 8.0, the resolution between AML and VAL decreased significantly due to the loss of charge.

In summary, the optimized conditions of the analysis were separation voltage 25 kV, injection time 10.0 s, column temperature 25°C, and 25 mM phosphate running buffer at pH 8.0.

Method Validation

For the purpose of calibration, a long series of mixed standard solutions, including AML and VAL in different concentrations, were examined under the optimized conditions. The method was found to be linear in the range of 1.0–35 and 1.0–350 mg/L, with weighted regression described in Equations 1 and 2, for AML and VAL, respectively:

$$PA = 465.34C - 1652.8, \quad r = 0.9999 \quad (1)$$

$$PA = 255.49C - 12756, \quad r = 0.9994 \quad (2)$$

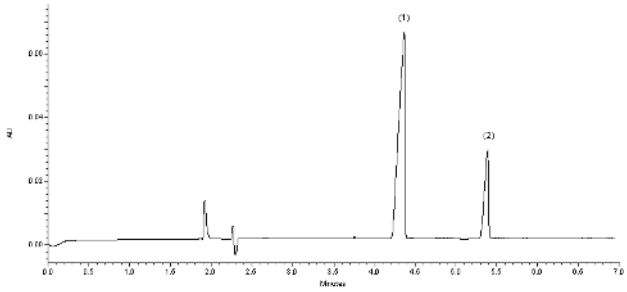


Figure 2. Electropherograms of 10 mg/L AML and 160 mg/L VAL in a tablet formulation (Exforge). Conditions: 10 s injection time, 25 kV voltage, 25°C, 25 mM phosphate electrolyte concentration, pH 8.0. Other conditions as described in *Experimental* section. Peak identification: (1) VAL and (2) AML.

where PA is the peak area, *C* is the analyte concentration in mg/L, and r is the correlation coefficient.

The LOD was calculated as the concentration of solute resulting in a peak height three times the baseline noise level, and the LOQ as 10 times the baseline noise level. The LOD for both AML and VAL was 0.03 mg/L, and the LOQ was 0.09 mg/L. The sensitivity of the method allowed analysis at the levels of AML and VAL possibly found in human plasmas. Good sensitivity was obtained by both the optimization of CE procedure and preconcentration performed by LLE for human plasma samples (preconcentration factor equal to 10).

The intraday accuracy and precision of the proposed method were examined for both pharmaceutical formulation and spiked human plasma samples. The obtained data are introduced in Table 1. The interday accuracy and precision were also examined (five analyses/day for 3 days) for pharmaceutical preparations and human plasma samples. For AML, interday accuracy was in the range of 85.5–91.7% and interday precision was 3.3–4.2%. For VAL, interday accuracy and precision were in the range of 86.3–92.5 and 2.7–4.4%, respectively. The proposed method gave good recovery for both pharmaceutical formulations and human plasma matrixes.

Analysis of Pharmaceutical and Human Plasma Samples

The optimized method was applied to real pharmaceutical samples (Exforge, Amlor, and Diovan). A typical electropherogram for Exforge is depicted in Figure 2. The figure shows successful separation and acceptable peak shape. Placebo sample was also analyzed by our CE method, and no peak was recorded, indicating good selectivity of the method. The method was also applied to a plasma sample spiked with the drugs under study and another plasma sample free from the drugs. The resultant electropherograms are shown in Figure 3.

Conclusions

For the first time, CE was used for simultaneous determination of AML and VAL in pharmaceuticals and biological samples. The adopted CE method is simple since it involves free electrolytes. It is also rapid because sufficient separation was obtained in a short time (<6.0 min). In addition, the CE method shows acceptable accuracy and precision as well as a wide range of linearity. The proposed method achieved good recovery and satisfactory resolution between AML and VAL. The LOD obtained allowed analysis at the levels of the drugs under study possibly found in human plasma. The proposed method was validated, and showed good performance with respect to selectivity. Therefore, the method is suitable to be applied for routine analysis in pharmaceutical and clinical laboratories or as a complementary technique to traditional methodologies.

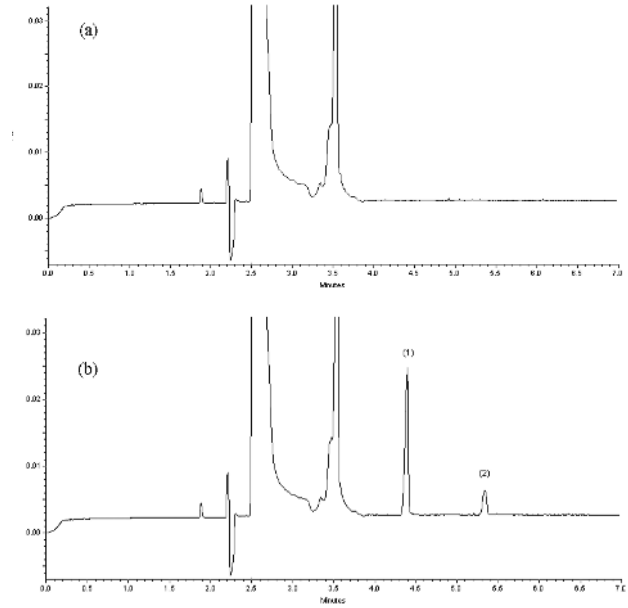


Figure 3. Electropherograms of (a) drug-free human plasma sample, and (b) human plasma sample spiked with 0.1 mg/L AML and 1.6 mg/L VAL obtained under the same conditions as for Figure 2. Peak identification: (1) VAL and (2) AML.

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